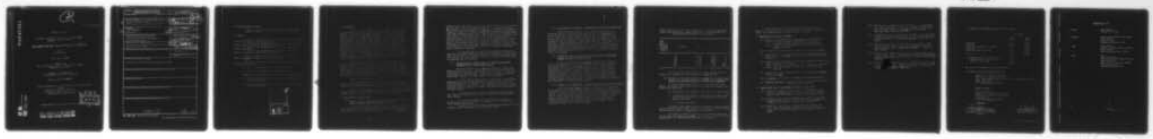
AD-A047 351 UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE (--ETC F/G 6/13
ATTEMPTS TO PRODUCE A VACCINE AGAINST SCHISTOSOMA MANSONI INFEC--ETC(U)
JAN 75 J PELLEGRINO DAMD17-74-G-9396

UNCLASSIFIED NL



END
DATE FILMED
-78
DDC

(2)

AD _____

REPORT NUMBER 6

"ATTEMPTS TO PRODUCE A VACCINE AGAINST SCHISTOSOMA MANSONI INFECTION IN MICE"

FINAL REPORT (2ND YEAR) AND REQUEST FOR RENEWAL (3RD YEAR)

by
J. PELLEGRINO

JANUARY, 15 - 1975

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract Nº 3A061102B 71Q
(Communicable Disease and Immunology)

Grant nº DAMD 17-74-G-9396

GRUPO INTERDEPARTAMENTAL DE ESTUDOS SOBRE ESQUISTOSSOMOSE
(GIDE)
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
UNIVERSIDADE FEDERAL DE MINAS GERAIS
30.000 Belo Horizonte, Brasil

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	(FINAL)
4. TITLE (and Subtitle) (6) Attempts to Produce a Vaccine Against Schistosoma mansoni Infection in Mice.	5. TYPE OF REPORT & PERIOD COVERED (9) Annual Progress Report, No. 6 1 Jun 74 — 31 May 75
6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s) (10) J. Pellegrino	8. CONTRACT OR GRANT NUMBER(s) (15) DAMD 17-74-G-9396
9. PERFORMING ORGANIZATION NAME AND ADDRESS Instituto de Ciencias Biologicas Universidade Federal de Minas Gerais Belo Horizonte, Brasil	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) 61102A 3A161102B71C 00.020 (17) 00
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Washington, D. C. 20314	12. REPORT DATE (11) 15 Jan 75
	13. NUMBER OF PAGES ~~14~~ pages (12) 10
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

406697

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

1 - TITLE OF RESEARCH PROJECT

"Attempts to produce a vaccine against Schistosoma mansoni infection in mice".

2 - SUMMARY

The study of schistosome antigens has usually been undertaken with one of these long-term aims in mind:

a) isolation and characterization of antigens with possible invoke acquired immunity in the vertebrate host,

b) isolation and characterization of antigens for immuno-diagnostic purposes,

c) studies of schistosome antigens toward an attempt to reveal the molecular architecture of the parasite.

Several attempts have been tried to achieve a "vaccine" against S. mansoni infection. All were unsuccessful.

Two novel attempts were discussed as to produce immunity in mice against S. mansoni:

a) the use of schistosomules as immunizing agent;

b) the use of cercarial tail as immunizing agent.

Therefore, the research objectives can be summarized as follows:

1. Attempt to induce immunity in mice using schistosomules as immunizing agent

2. Attempt to induce immunity in mice using tail of cercariae as immunizing agent.

3 - INTRODUCTION

Two new and novel aproaches are now proposed with the aim to induce acquired immunity in schistosomiasis. Groups of mice will be exposed to 500-1,000 S. mansoni cercariae (L.E. strain) by the tail immersion method. Shortly before exposure, and at different periods after exposure, the animals will be treated with compound U.K. 3883 (Richards and Foster. 1969. Nature 222: 581-582) which effectively kills migrating schistosomules (Pellegrino. 1970. Unpublished data). Therefore, it is expected that schistosomules at different stages of development - before maturing into adult worms - be destroyed and so produce an antigenic stimulus followed by acquired immunity through the liberation of functional antigens. Exposed and "cured" animals will be challenged with 200 S. mansoni cercariae (L.E. strain) 15, 30, 60, and 90 days after the first and aborted infection. Control animals will be included to check the effectiveness of U.K. 3883 in curing the initial infection (exposure to 500-1,000 cercariae) and to serve as comparison to the challenge infection (exposure to 200 cercariae).

The following criteria will be used for the assessment of acquired immunity: a) reduction in worm burden; b) abnormal development of schistosomes; c) changes in the normal pattern of egg-laying (oogram); d) changes in the distribution of worms within the hepatic-portal system. Technical details and pertinent references are found in the reviews by Stirewalt (1963. Exp. Parasit. 13: 18-44), Pellegrino and Katz (1968. In Advances in Parasitology, vol. 6, pp. 233-290) and Smithers and Terry (1969. In Advances in Parasitology, vol. 7, pp. 41-93).

Preliminary experiments seem to favour this idea.

Another approach, not yet attempted, is to use the tail of cercariae as an immunizing agent. It is known that the tail is casted off during the normal process of infection and does not enter the parasite.

Preliminary experiments have shown that when cercariae (tail and body) are injected by the subcutaneous route and a second exposure is made exposing the tail of mice, only a few worms could be recovered.

4 - EXPERIMENTAL WORK UNDERTAKEN

4.1 - Activity of some tetrahydro- and pyrazinoquinolines against early developing forms of Schistosoma mansoni

The antischistosomal activity of Pfizer's tetrahydroquinolines (U.K. 3645, 3954, 4885, 3883, and 4271) and pyrazinoquinolines (U.K. 4210, 5076, and 5944) on the early developing forms of S. mansoni was

evaluated using the method of Pereira, Pellegrino et al. (1974. J. Parasit 60: 723-725). Albino mice, weighing about 20 g, were injected intraperitoneally with 150 cercariae of S. mansoni (L.E. strain, Belo Horizonte). Within a few hours all recovered organisms were schistosomules (Eveland, 1972, Expl. Parasit., 32: 261-264). Three hours after inoculation, groups of 10 mice were treated with a single dose of drug, via i.m. or p.o., the doses varying from 50 to 250 mg/kg. All animals were sacrificed by cervical fracture, one week later. The skin was removed from the abdomen and 5 ml of isotonic saline injected into the peritoneal cavity. After opening the peritoneal cavity, the liquid was collected in a Petri dish. Additional saline was used to wash carefully the abdominal viscera. Larvae were concentrated by centrifugation for 1 min at 1,000 rpm and counted under a dissecting microscope.

The results obtained showed that no larvae (schistosomules) were found in peritoneal washings from mice treated with U.K. 3645, 3954, 4210, 4271 and 5076. A significant reduction of larvae was observed with U.K. 5444 and U.K. 3883. Compound U.K. 4885 did not reduce larval counts in comparison with the control.

4.2 - Factors affecting surface changes in intact cercariae and cercarial bodies of Schistosoma mansoni

Tails were removed from S. mansoni cercariae by cooling plus whirling in a vortex mixer. Surface changes were induced in 80% of the bodies following incubation in TC-199 or Hank's BBS at 27°C for 140 min. In contrast, only less than 20% of intact cercariae showed the surface changes when incubated in TC-199 medium for the same period.

At 37°C, 100% of surface changes were observed in tail-less cercariae in 80 min. Intact cercariae also showed a greater tendency to water sensitivity at 37°C although this response was dependent on the incubation medium; in 90 min 80% of the cercariae in TC-199 being water sensitive compared to only 30% of those in Hanks's BBS and less than 10% of those in water.

At pH 6.25, surface changes of tail-less cercariae occurred very slowly and increased in rate with increasing pH in both Tris and Hepes buffers.

EGTA and EDTA produced some enhancement in the rate of acquisition of water sensitivity. An excess of Ca^{++} in the incubation medium retarded the process.

Surface changes in tail-less cercariae were independent of the incubation medium and occurred even in distilled water.

4.3 - A new approach for screening prophylactic agents in schistosomiasis

Schistosomula were obtained in the peritoneal cavity of mice previously injected intraperitoneally with cercariae of S. mansoni. The number of recovered larvae from peritoneal washings was about 30% after 1, 2, 5, and 8 days of injection. Drugs were given to groups of 20 mice 3 hours after the cercarial injection (oxamniquine) and continuing for 4 consecutive days for triostam. Animals were sacrificed 8 days after starting treatment. Schistosomula were counted under a dissecting microscope. Oxamniquine as well as triostam were used to evaluate the screening method. Oxamniquine-treated mice showed no larvae; triostam group presented 5% of recovered larvae against 45% showed by the control. The results obtained agree with previously described effects of these drugs on early developing stages of S. mansoni.

4.4 - Biochemical markers to trace the transformation of Schistosoma mansoni cercariae to schistosomules

In the transformation of S. mansoni cercaria to schistosomule there is an elimination of the ATEE-hydrolysing proteases to about 50% of the original specific activity level in 40-80 minutes. Histochemical examination of the pre- and post-acetabular cercarial glands disclosed similar loss of material when stained by alizarin and PAS. The ratio of carbohydrate/protein also decreased to about 50% of its original value. Extracts of cercariae, tails, and bodies submitted to electrophoresis in deoxycholate-containing borate buffer pH 9.5 disclosed three PAS-stained bands, identified from the anode to the cathode as pre-acetabular gland contents, surface coat and glycogen. The effacement of these bands occurred in the course of the cercarial transformation.

4.5 - Studies on the protection of Cebus monkeys with irradiated cercariae

Three adult Cebus apella monkeys were infected, by percutaneous route, with 500 irradiated cercariae (2,500 r, ^{60}Co bomb). No schistosome eggs could be found in rectal snips taken by mucosal curettages up to 3 1/2 months. The monkeys were then reinfected with 2,500 r-irradiated cercariae. Two and a half months after the second infection the 3 monkeys - without eggs in rectal tissue - were challenged with 200 normal cercariae (L.E. strain, Belo Horizonte) by the percutaneous route. Serial mucosal curettages were performed at different times after exposure. Results obtained are shown in the Table on next page.

Number of viable eggs per gram of rectal tissue in 3 monkeys previously exposed with irradiated S. mansoni cercariae and challenged with 200 normal cercaria by the transcutaneous route.

Days after exposure to normal cercariae (200) / Monkeys	1	2	3
61	1146	1000	0
91	1638	2529	29
152	200	10031	0
228	1696	11838	0
438	4808	2392	2428
541	2469	8704	3400

It is interesting to remark that all three monkeys took the infection, in contrast that would happen with Rhesus monkeys.

4.6 - Treatment of infected patients (S. mansoni) in an endemic area: action of an immunostimulant drug associated with hycanthone

In a small village (Tuparecê) in the North of Minas Gerais, Brazil, wo groups of patients with chronic schistosomiasis mansoni were treated with hycanthone:

a) 104 patients with hycanthone (2.5 mg/kg, i.m., single dose)

b) 81 patients with hycanthone (2.5 mg/kg, i.m., single dose) plus one tablet of 80 mg of an immunostimulant (J.A.) per month for 12 months

c) 51 patients not treated

A parasitological control (quantitative Kato's method), carried out in group (a), one year after treatment, revealed that 19.1% of treated patients continue passing S. mansoni eggs in their feces.

The control of group (b) revealed, in 66 patients followed up for one year, 21.2% passing S. mansoni eggs. It can be concluded that the association of the immune stimulant drug was not effective.

Stool examination in the control group (c) showed that all patients, but one, presented a decrease in the number of eggs per gram of feces. The explanation for this finding is not yet known.

5 - EXPERIMENTAL WORK UNDER DEVELOPMENT

5.1 - Treatment of infected patients (S. mansoni) in endemic areas
5.1.1 - Studies on concomitant immunity in reinfection u quantitative stool examination (Kato-Katz method)
5.1.2 - Studies on the correlation between IgE levels and S. mansoni infection

5.2 - Studies on RES in mice experimentally infected with S. mansoni.

5.3 - Continuation of the studies on "vaccines" prepared with sonicated larval forms of S. mansoni and by killing chemically early schistosomules.

5.4 - Studies on new formulations of niridazoli, hycanthone, oxamniquine, Roche tiophenes and EMIAY 8440.
5.4.1 - Cutaneous route (curative as well as protective activity)
5.4.2 - Nasal route (spray): curative as well as protective activity

5.5 - Activity of human and animal sera on schistosomules (S. mansoni) obtained in vitro.

5.6 - S. matheei: a new model for studying antischistosomal agents.

5.7 - Mastomys natalensis: a new model for immunological investigation of antischistosomal agents.

6 - PAPERS SUBMITTED FOR PUBLICATION (SUPPORTED BY THE GRANT)

6.1 - HOLANDA, J.C. & PELLEGRINO, J. 1974. Infectivity of schistosomula (S. mansoni) recovered from the skin and the lung of infected mice. Rev. Inst. Med. trop. São Paulo, 16: 127-131.

6.2 - PELLEGRINO, J., KATZ, N. & COELHO, P.M.Z. 1973. Reinfection of Cebus monkeys, experimentally infected with S. mansoni, after successful chemotherapy. J. Parasit. (in press).

6.3 - PELLEGRINO, J., KATZ, N., VALADARES, T.E. & CARVALHO, H.C. 1973. Loss of resistance to hycanthone and oxamniquine in experimental schistosomiasis. Ann. Soc. Trop. Med. & Hyg. (in press).

6.4 - HOLANDA, J.C., PELLEGRINO, J., GAZZINELLI, G. & RAMALHO-PINTO, F.J. 1974. Infectivity for mice of cercariae, schistosomula and intermediate forms of S. mansoni obtained in vitro. Rev. Inst. Med. trop. São Paulo (in press).

6.5 - RAMALHO-PINTO, F.J., GAZZINELLI, G., HOWELLS, R.E. & PELLEGRINO, J. 1974. Factors affecting surface changes in intact cercariae and cercarial bodies of Schistosoma mansoni. Parasitology (in press).

6.6 - PELLEGRINO, J., PEREIRA, L.H., MELLO, R.T. & KATZ, N. 1974. Activity of some tetrahydro- and pyrazinoquinolines against early developing forms of Schistosoma mansoni. J. Parasitol., 60: 723-725.

6.7 - PEREIRA, L.H., PELLEGRINO, J., VALADARES, T.E., MELLO, R.T. & COELHO, P.M.Z. 1974. A new approach for screening prophylactic agents in schistosomiasis. Rev. Inst. Med. trop. São Paulo, 16: 123-126.

6.8 - OLIVEIRA, [illegible]C., FIGUEIREDO, E.A., GAZZINELLI, G., HOWELLS, R.E. & PELLEGRINO, J. 1974 - Biochemical markers to trace the transformation of Schistosoma mansoni cercariae to schistosomules. J. Comp. Biochem. Physiol. (in press).

7 - BUDGET FOR THE THIRD YEAR (JUNE 1975 - MAY 1976)

U.S. Dollars

	From Grantee	From Grant
Salaries *	15.000	7.200
Equipment	2.000	500
Expendable equipment and supplies	1.000	1.000
Travel **	-	2.000
Books and publication costs	2.000	500
T O T A L	20.000	11.200

* Principal investigator (50% time) 5.000
Research assistant (25% time) 1.000
Technician (50% time) 1.200
7.200

** To attend scientific international meetings

8 - NAME OF INSTITUTE TO EXECUTE CONTRACT

Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais
Grupo Interdepartamental de Estudos sobre Esquistossomose (GIDE)
Caixa Postal 1404
30000 Belo Horizonte, Brasil

Mailing address:

Dr. J. Pellegrino
Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais
C. Postal 1404
30.000 Belo Horizonte, Brasil

Signatures:

Prof. Roberto Alvarenga
Director, Institute of Biological Sciences

Dr. J. Pellegrino
Principal Investigator

Belo Horizonte, January 1975

DISTRIBUTION LIST

4 copies	HQDA (SGRD-AJ) Washington DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, Maryland 20014